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# Proceedings of the Salmonella Seminar

Held at
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND
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Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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# PREFACE

The U.S. Department of Agriculture has had an interest in salmonellosis since 1885, when Dr. D. E. Salmon, first Chief of the Bureau of Animal Industry, isolated and identified the enteric organism that was given the species name Salmonella choleraesuis.

Salmonella choleraesuis became the first of a large and growing genus of the disease-producing enterobacteriaceae that plague livestock, poultry, and man. By the turn of the century, the Salmonellae that cause fowl typhoid and pullorum had been studied. In the first decade, their cycle of transmission was known and a diagnostic antigen obtained. During the period 1920-1930, eradication programs against S. gallinarum and S. pullorum were started in a few of the Eastern States. During the past quarter of a century, a control program for these two Salmonellae has been effective in flocks participating in the National Poultry Improvement Plan and the National Turkey Improvement Plan. The diseases produced by these organisms caused the greatest threat to the poultry industry at that time. The success of the National Poultry Improvement Plan and the National Turkey Improvement Plan against pullorum disease and fowl typhoid is a monumental achievement obtained by the cooperative efforts of industry and State and Federal agencies.

With the decline in losses due to S. pullorum and S. gallinarum, losses due to other serotypes of the Salmonellae became more apparent. To meet the need of animal disease diagnostic laboratories for serotyping, the Animal Disease Eradication Division established a national Salmonella serotyping facility in 1957 for typing cultures from nonhuman sources. During the first six and one-half years of its operation, this laboratory serotyped 13,457 cultures of Salmonella and found 11,598 of these were of serotypes other than S. pullorum and S. gallinarum. With the exception of S. typhimurium infections in turkey breeder flocks, little or no organized effort has been exerted against the other serotypes.

During this period, many studies indicated that contaminated animal feeds may be a source of Salmonella infection. In an attempt to determine the incidence of Salmonella contamination in animal feeds and animal byproducts, the Animal Disease Eradication Division surveyed the reports of the diagnostic laboratories, universities, experiment stations, and private industry in 31 States. This survey was published in November 1961, and revealed that 5,712 samples of these products had been tested with a contamination rate of 12.5 percent.

Members of the National Renderers Association and the National Fisheries Institute cooperated with several Government agencies in 1963 to develop separate "Recommended Sanitation Guidelines" for processing of their products (to be used in animal feeds). Included in the guidelines was a provision for periodic sampling and testing of the finished product for the presence of Salmonella.

Many Divisions within the Agricultural Research Service and the Agricultural Marketing Service have studied the Salmonella problem in line with their research or regulatory responsibility, but many questions are still unanswered. Recent severe outbreaks of salmonellosis in the human population has created a greater stimulus for the Agricultural Research Service to attack this problem.

In planning the Salmonella activities for the Animal Disease Eradication Division, many questions confronted the staff members. A few of the seemingly more pertinent questions are:

1. How serious is Salmonella contamination in animal feeds and feed ingredients? And what approach should be taken to correct the situation?

2. Is salmonellosis in cattle, swine, and other domestic animals economically significant? And will it become a greater problem as dairy herds and feed lots increase in size and concentration of animals?

3. Studies have indicated that the infection rate in slaughter swine increases as they go through concentration points (Niven, et al., American Meat Institute Federation; and Galton, et al., Communicable Disease Center, U.S. Public Health Service). Is this significant and what factors are involved? Can they be corrected?

4. What role do insects, rodents, snakes, and other wildlife play in the overall Salmonella picture?

5. If it appears that additional research will be needed, what particular phases should receive priority?

6. Salmonella surveys and increased activity will require additional laboratory support on the local level. Will the State diagnostic laboratories be able to provide this service? What part will the commercial laboratories play?

7. It appears that a national report of all laboratory confirmed cases of salmonellosis in livestock and poultry should be developed as a foundation for epidemiological studies and future programs. Will it be possible to obtain these laboratory reports and how should they be submitted?

More recent severe outbreaks of salmonellosis in humans have emphasized the need for continued attention of Salmonellae, and their cycle of transmission from animal to man to animal.

The Animal Disease Eradication Division has been intensively studying the contamination of animal feeds and the farm incidence of salmonellosis, and working with various interested industry groups in introducing measures that will alleviate or correct various phases of the Salmonella problem. We are planning to do more. In connection with our future work, this Division sponsored the following seminar for the Hyattsville staff members to assist them in focusing and refining the Salmonella activities of this Division. The knowledge and experience of the seminar participants is duly recognized. We believe they represent the best in the veterinary profession on this subject. And their many excellent recommendations and suggestions are deeply appreciated. From this we hope to build an even better attack on the Salmonella problem.

# GREETING TO PARTICIPANTS

By
F. J. Mulhern*

We want to express our appreciation to the guest lecturers for coming here to Hyattsville, Maryland, to instruct the Animal Disease Eradication Division staff and their guests from other Agricultural Research Service Divisions on possible approaches to defining and correcting the Salmonella situation.

We have been confronted recently with problems in Agriculture in which we are required to act on a "crash" or "crisis" basis. We are hoping that bringing the people together for the Salmonella Seminar will not only identify and point out ways of alleviating a potentially serious problem but will also avoid unpleasant situations from developing in the future. We want to avoid a situation developing in animal and poultry feeds, and food products from them, similar to the cranberry or stilbestrol scare. This we are trying to avoid by working closely with research workers, State officials, and industry groups.

We are particularly interested in new ideas or innovations that the scientists may be able to suggest for a novel approach to disease control. For example, is it practical or even possible to utilize bacteriophages in the eradication of Salmonella and other disease-producing agents? We know something of the action of the bacteriophage, however, no one has made practical application of it except for laboratory identification purposes. We would like to know, for example, whether bacteriophages could be used in nature or on an infected premise to cause disease in Salmonella bacteria and thus used as a tool in the eradication or at least in the control of disease.

We do not know the answer to this and other pertinent questions we have on salmonellosis. We need your group's recommendations on what should be done to use this approach on other lines of attack on the Salmonella problem. In summary, we need to have defined for us what more can be done with the knowledge we now have and in what areas we need to concentrate needed research work.

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* Director, Animal Disease Eradication Division, ARS, U.S. Department of Agriculture.

# PURPOSE OF MEETING

By
R. E. Omohundro*

We wish to take this opportunity to greet all of you this morning. We deeply appreciate your being able to work with us and participate in the Salmonella Seminar, and assist and guide the Animal Disease Eradication Division in attacking the various aspects of the Salmonella problem. We would like your assistance and guidance on the following problems:

1. Advice and recommendation of technical people on what steps they think we should take toward the Salmonella problem in all livestock and poultry.

2. What approach should the Agricultural Research Service take on the contamination in feeds by Salmonella.

3. Hospital-associated outbreaks of Salmonellae in recent months have created much public interest and the human investigators definitely blame livestock and poultry sources, whereas we in the animal field are inclined to incriminate man. We need to get factual information in order to assist in correcting the problem as well as to prevent loss of domestic as well as foreign markets.

4. The Animal Disease Eradication Division, and particularly this group, is interested in salmonellosis of all species of livestock and poultry.

   a. We need to know considerably more about this condition in cattle. Large concentrations of feeder cattle are now commonplace (4000-5000 head).

   b. Swine concentration points along the slaughter route have been proven to be heavily contaminated. Dr. C. F. Niven, formerly of the American Meat Institute Foundation, has published on this.

5. Surveys of Salmonellae will require laboratory support on local level. Already there are indications of interest on the part of commercial laboratories. We hope States will make their own arrangements for the culture work and furnish us with their results. The National Animal Disease Laboratory will do typing.

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6. The Animal Disease Eradication Division will assume the responsibility for analyzing, summarizing, and disseminating the information back to all concerned.

7. If further research is needed, what particular phases and in what sequence?

We appreciate each of you taking time from your valuable work to come and consider the overall problem of salmonellosis. Needless to say, the Animal Disease Eradication Division is most appreciative of your advice and counsel.

# SALMONELLOSIS IN POULTRY AND SWINE

By
B. S. Pomeroy*

Salmonella infections of poultry and livestock continue to be important problems in the United States. Over 800 Salmonella serotypes have been identified and over 100 serotypes have been isolated from poultry. Salmonellae are recognized as potential pathogens, and it has been established that there is considerable variation in the pathogenicity of the various serotypes. Also, the host range may be limited as in the case of Salmonella pullorum or quite broad as in the case of S. typhimurium that has been found in more than 40 different species of animals.

During the past 30 years in Minnesota there has been considerable interest in salmonellosis in livestock and poultry particularly turkeys. Because the turkey is highly susceptible to Salmonella and Arizona infections, it has served as an excellent means of establishing the Salmonella and Arizona flora for an area. Approximately 60 serotypes of Salmonella have been isolated from groups of turkeys submitted from 1379 field outbreaks over a span of 30 years. About 30 percent of the isolations have been S. typhimurium. Some types have been consistently isolated over this period, others have been isolated only once or twice. Table I gives the 10 most common Salmonella types encountered over this period as well as the Arizona group.

If we examine the current situation of the past two years (1962-1963), other types of Salmonellae are emerging in significant numbers. Twenty-two serotypes were isolated from adult turkeys that have reacted to group B and D antigens. For example, many flocks have been tested and the following serotypes have been isolated:

| Isolations | Number of Flocks |
|---|---|
| S. heidelberg | 47 |
| S. saint paul | 28 |
| S. typhimurium | 25 |
| S. chester | 22 |
| S. muenchen | 15 |
| S. newport | 11 |
| S. typhimurium var. copenhagen | 8 |
| S. bredeney | 8 |
| S. enteritidis | 7 |
| S. schwarzengrund | 7 |
| S. derby | 6 |

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In addition, seven flocks were found infected with Arizona organisms by using an Arizona antigen.

In this same period, 18 serotypes were isolated from young poults submitted to the diagnostic laboratories. Fifty-eight flocks were found infected with Salmonellae involving over 500,000 birds, for example:

| Isolations | Number of Flocks |
|---|---|
| S. typhimurium | 9 |
| S. typhimurium var. copenhagen | 6 |
| S. saint paul | 6 |
| S. worthington | 5 |
| S. infantis | 5 |
| S. schwarzengrund | 3 |
| S. derby | 3 |
| S. tennessee | 3 |
| S. thompson | 3 |
| S. chester | 2 |
| S. newport | 1 |
| S. heidelberg | 1 |

In addition, 19 flocks were found infected with Arizona organisms involving 150,000 birds. The mortality of the birds infected with Salmonellae varied from 5 percent to a high of 50 percent. During 1962-1963, 14 feed samples were examined and 7 different Salmonella serotypes were isolated from 11 samples. Previous experience involved approximately 1,000 samples of feed ingredients originating from 22 states. Fifty-two Salmonella serotypes were isolated from 175 samples.

It is recognized that in poultry there are several modes of spread and in some Salmonella serotypes one method may be more important than another. Modes of spread may be as follows:

1. Ovarian transmission
2. Egg shell penetration
3. Incubator contamination
4. Contaminated feed
5. Infected rodents and snakes, etc.
6. Cats, dogs, and other farm animals
7. Infected premise
8. Contaminated litter or water
9. Contaminated transportation vehicles
10. Infected feral birds

In a recent study, 11 of the common Salmonella serotypes isolated from adult turkeys were studied to determine their relative pathogenicity in young poults. The studies with S. saint paul illustrate the variation that may

occur in pathogenicity. Ordinarily, 0.05 ml. to 0.1 ml. of 24-hour-beef-infusion-broth culture of a Salmonella serotype when given orally will cause some mortality in day-old poults. S. saint paul on three repeated groups of poults did not produce any mortality, but the poults became infected. The Salmonella isolated from the third group was given intraperitoneally to another lot of poults and high mortality occurred. The isolate from this group was again tried orally in young poults. On this trial, 20 percent mortality occurred and the following two experimental groups had a mortality of 20 and 40 percent. Under field conditions, outbreaks involving S. saint paul have resulted in average mortality of 10 percent. Because the Salmonella organisms localize in the digestive tract, many birds become intestinal carriers. The organism may become localized in the digestive tract for weeks or months and serve as a potential source of contamination of poultry meat and egg products during processing. The public health hazard may be eliminated by providing poultry food products free of Salmonella.

TABLE I

INCIDENCE OF PARATYPHOID AND ARIZONA INFECTIONS IN YOUNG TURKEYS
IN MINNESOTA 1935 - December 31, 1963

| | 1935 to 1943 | 1944 to 1950 | 1951 to 1955 | 1956 | 1957 | 1958 | 1959 | 1960 | 1961 | 1962 | 1963 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. S. typhimurium | 79 | 161 | 105 | 12 | 11 | 8 | 4 | 7 | 4 | 2 | 4 | 397 |
| 2. S. anatum | 13 | 59 | 28 | 5 | 8 | 7 | 5 | 8 | 1 | 2 | 1 | 137 |
| 3. S. meleagridis | 9 | 38 | 12 | 7 | 8 | 1 | 1 | 0 | 0 | 0 | 0 | 76 |
| 4. S. derby | 10 | 45 | 11 | 4 | 3 | 1 | 0 | 1 | 0 | 0 | 1 | 76 |
| 5. S. give | 8 | 44 | 3 | 1 | 3 | 6 | 1 | 1 | 0 | 0 | 0 | 67 |
| 6. S. newport | 3 | 16 | 11 | 7 | 5 | 4 | 4 | 5 | 2 | 1 | 1 | 59 |
| 7. S. bredeney | 14 | 26 | 5 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 49 |
| 8. S. oranienburg | 6 | 28 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 38 |
| 9. S. california | 0 | 24 | 6 | 4 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 40 |
| 10. S. kentucky | 0 | 18 | 2 | 1 | 2 | 6 | 0 | 2 | 0 | 0 | 0 | 32 |
| 11. Arizona types | | 40 | 67 | 13 | 11 | 9 | 2 | 3 | 3 | 4 | 8 | 160 |

# SALMONELLOSIS IN CATTLE

By
R. V. Lewis*

This Salmonella Seminar would seem to be most apropos, for apparently Salmonella infections in cattle in California are becoming more frequent. At least, they are being diagnosed more often now than in past years.

## Sources of Data

My discussion will be confined to only cattle salmonellosis, and any data that I have is drawn from the records of and experiences in the State of California Department of Agriculture diagnostic laboratories. Our State has six of these laboratories; two of them are devoted to poultry only and the remaining four are general laboratories that accept both poultry and other classes of livestock. These four general diagnostic laboratories service the southern, central, and northern parts of the State. In California, all livestock other than poultry must be submitted to the laboratories either by or through a veterinarian and he is the one who receives our written report. Therefore, all cattle cases would fall within a practicing veterinarian's perusal at one time or another. We, as a laboratory, seldom make recommendations for treatment; we leave the handling of the case to the practicing veterinarian. Of course, we often discuss the case with him by telephone should he desire to do so.

## Incidence and Types

Regarding the incidence and types of salmonellosis, the few statistics I have to present have been obtained for the last two years (1962 and 1963) from the annual report that each laboratory submits to our Sacramento headquarters. Whenever a Salmonella is isolated, we submit a culture to the State Public Health Laboratory in Berkeley for typing, and usually we receive this information from them about one week after we send them the culture. Thus, all Salmonellae isolated in our laboratories - both poultry and general diagnostic - are typed, and this is reported to the Sacramento office when received from the State Public Health Laboratory and again in our annual report.

With regard to the incidence of salmonellosis for the last two years, we found that there was a total of 276 Salmonella isolations from cattle in 1963 and 122 in 1962, in our four laboratories. Salmonella typhimurium is most commonly isolated. There were 134 isolations of this type in 1963, and 70 in 1962. The next two most often isolated types are S. dublin and S. newport. We found that 91 S. newport and 37 S. dublin types were recovered in 1963, while in 1962 there were 31 recoveries of S. dublin and 10 S. newport types.

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There were ten other types of Salmonellae isolated and typed, but few with any consistency. Most of these ten miscellaneous serotypes were recovered on so-called "routine culture," not necessarily from a suspected enteric infection. Whenever any intestinal tissue or contents are submitted as specimens from a case, they are routinely cultured for Salmonella, even though the case is obviously leptospirosis, for example. In other words, in those cases that are basically enteric in involvement, either S. typhimurium, S. newport, or S. dublin was almost always the one involved whenever a Salmonella was isolated.

Types of Cattle Operations

We can divide the different kinds of cattle operations in which Salmonellae have been involved into three types.

1. Dairy Cows

(a) Raw Milk - No Calves

There have been only a very few cases in which the adult milking cow has been the primary and only problem. The typical story of this type of operation has been described and discussed in the 1959 issue of the Proceedings of the United States Livestock Sanitary Association. This outbreak occurred in southern California and caused 47 human S. dublin cases. This was a certified raw milk dairy. No calves were raised or kept on the premises. There had been no illness in the herd. The most probable source of the offending bacteria to the humans was fecal contamination of the milk, for on October 28, 1958, the bacterial count for that day suddenly increased considerably, and the report of the bacteriologist was that there were too many coliforms to count. On October 29, the following day, the first human patient showed clinical illness. All of the human cases became ill within the next five days and S. dublin was isolated from a significant number of them. Through fecal sampling of the herd, which numbered 398 animals, three cows were identified as being shedders of S. dublin. Before the herd was considered safe for normal operations, about 1600 fecal cultures were made. The three shedder cows were removed from the herd and sampled at 4- to 24-day intervals, five times. In two of the animals, S. dublin was recovered quite consistently, while in the remaining cow it was recovered only at the first sampling. At slaughter of these animals, S. dublin was isolated from the bile of the two consistent shedders and not from the remaining cow. As previously mentioned, only a bare handful of cases of this sort have occurred and it would go unnoticed but for the human infections resulting from the consumption of contaminated raw milk.

(b) Dairy with Calves

We also have the more conventional dairy--that does not produce certified raw milk and also raises its own calves and heifers for replacement stock. The adult stock may or may not show signs of clinical illness. In an outbreak of S. typhimurium in a dairy herd that was reported in the "California Veterinarian" of January 1956, the adults, as well as the heifers, were visibly affected. This was a dairy herd located in the San Joaquin Valley, north of Fresno, consisting of 52 milking cows and 24 heifers. Overnight, milk production had dropped ten cans of milk. There was a severe diarrhea, watery and with some blood, in both the adults and heifers. There was a decided temperature increase in the animals. The herd was on pasture until five days prior to illness, when they were placed on green, chopped alfalfa. The significance of this dietary change is not known. S. typhimurium was recovered by fecal sampling and the same type of bacteria was isolated from all tissues submitted from a heifer that died. Three weeks after the beginning of the trouble S. typhimurium agglutination tests were run on serums from 34 animals.

To the O antigen:

| Negative | 1:25 | 1:50 | 1:100 | 1:200 |
|---|---|---|---|---|
| 1 | 3 | 9 | 17 | 4 |

To the H antigen:

| Negative | 1:25 | 1:50 | 1:100 | 1:200 |
|---|---|---|---|---|
| 7 | 23 | 3 | 1 | 0 |

There was a favorable response after treatment in most animals in 72 hours, and full milk production was obtained in approximately two weeks.

The serological diagnosis of salmonellosis, as just described, has been used to some extent in our State, but not a great deal has been done along this line. Our Sacramento laboratory has used the pullorum and typhimurium tube antigens, that we employ in our turkey testing programs, in cattle herds suspected of suffering from Salmonella infections. Significant titres have been obtained in adults infected with S. dublin and S. typhimurium if sufficient time has elapsed after infection. Research along this line would be in order in chronically infected, self-contained herds that have a continuing problem with calf scours due to Salmonella.

(c) Dairy with Calves

To continue the descriptions of types of dairy herds in which Salmonellae have been a problem, we also see the herd in which the adult is apparently unaffected but only the calves and heifer replacements become ill. A typical description will be found in the October 1954 issue of "Veterinary Medicine." The large herd of 1200 milkers appeared normal, but 50 two-month-old calves became ill, showing inappetence, depression, and diarrhea. Seventeen animals died after a period of illness of from three to seven days. Drug treatment and changes in sanitation appeared to stop the losses.

2. Feeder Cattle

Feed lots are another segment of our cattle industry in which disease and death of animals due to salmonellosis has occurred. There has been a noticeable increase of this disease in the last three years in feed lots. S. typhimurium and S. newport are the principal types involved. The disease usually first manifests itself with an uncontrollable diarrhea. If this scouring is not controlled, depression, dehydration, and death eventually occur. By this time the disease has become septicemic, and the offending bacteria can be recovered from organs other than the intestine and related lymph nodes. Respiratory involvement is not a constant feature. In many of our feed lot facilities, clostridiosis, pasteurellosis, and other bacterial and viral infections are found concomitantly with the salmonellosis. It has also been observed that in the majority of feed-lot outbreaks, cattle involved have been imported from the Southern States area. This would include Texas and Mexico and any States to the east of this region. Cattle brought in as feeders from other parts of California or other States in the West or Northwest, have not been plagued with this disease of salmonellosis as have the so-called "Texas" cattle. It would also seem that the imported feeders carry the Salmonella in their system some place and, due to the stresses inherent in gathering, assembling lots, and shipping, the carrier state is disturbed and a disease outbreak results. It seems unlikely that the imported animals first contact the disease when they enter the feed lot. If a new lot of feeders is put into the same pen that just previously contained an infected lot, we have found that the new lot will suffer little or no loss from salmonellosis. In this case, there was no attempt at cleaning or disinfection. Quite likely because the animals are fed and watered in equipment raised off the ground, there is little likelihood of their ingesting overpowering doses of the Salmonella.

Many of our feed lots are located in areas that do not get very much rain, but plenty of sunshine. These two climatic factors have a deleterious effect on bacteria. But these two environmental factors have disadvantages, however, in the fact that they encourage

dryness and dustiness, and the latter situation certainly aggravates respiratory problems that may then enter into rousing a latent salmonellosis problem. Infectious bovine rhinotracheitis, pasteurellosis, and mycoplasmosis are the three main respiratory diseases that we find in feed-lot cattle and that are enhanced by dust inhalation. Clostridium septicum or Cl. sordellii are often isolated from the tissues from which we isolate Salmonella. The association of these isolates, if any, is not known. Treatment has been more or less satisfactory. Our laboratories perform sensitivity tests on the Salmonellae recovered. This gives the attending veterinarian information as to what drugs not to use and those drugs that may be efficacious. The difficulty in treatment revolves around the fact that the infected animals are not noticed until they are quite ill. One must rely heavily on the cowboy riders to spot the sick animal and, naturally, this ability to pick out these animals varies with individuals. If the clinically ill animal is septicemic by the time it is discovered, most treatments are to no avail.

Thus, biological prevention is being practiced. Autogenous bacterins have been used with apparently good results. Two bacterin injections have been found to be satisfactory, with no less than a five-day interval between them. The cattle companies at the point of origin have cooperated well and have given the feeders one injection prior to shipment. Then the animals are given another inoculation as soon as they are received at the feed lot. The immunity obtained seems to carry the animals long enough so they get through the early danger period.

3. Calf-Raising Ranches

There are ranches in California devoted exclusively to raising day-old calves. These calf-raising ranches may be unique because the dairies of southern California, from which the day-old calf is obtained, are somewhat different from the usual dairy. The number of milking cows in a herd may be three or four hundred and more. Milking this number is practically a twenty-four hour job, and the milkers work in shifts. In certain areas, these dairies are located side by side along the road. There is no pasture for the animals. When they are through milking, the animals are turned out on a dry lot and fed hay that has been shipped in, as it is not raised on the ranch. No calves or replacement heifers are raised on the dairy ranch, and all replacements are purchased as springing heifers. When it is no longer economically feasible to retain the adult milking cow, she is sold to slaughter. As you can understand, this type of operation produces many surplus calves. At one time, these calves were a drug on the market and were either destroyed or given away. However, because of competition, these calves are now sold either to calf buyers for salesyard disposal or directly to people whose business it is to raise day-old calves.

These calves are raised either for vealing or baby beef purposes, or for heifer replacement stock. This exclusive calf-raising enterprise has spread from southern California up to the San Joaquin area, around Fresno. The disease problems that ensue from this mode of operation are the same in both southern and central California. Since these calves are gathered practically when they are dropped, they have little or no opportunity for colostral ingestion. Thus, they have no maternal antibody protection. They are transported in trucks, along with animals from other ranches, either to sales or to ranches. The conditions are ideal for these calves to be exposed to the myriad of bacterial flora of their fellow calves and, also, to the flora that is still present in the excrement of the day-old calves who occupied the salesyards or trucks the previous day or week. In addition to this, these defenseless animals may be hauled in any type of vehicle for 30 to 400 miles. Obviously, it is no wonder that there may be even a 100-percent mortality rate for these calves. Actually, the wonder is that any animals survive this rigorous regime. When the calves finally arrive at the home ranch, they may or may not be segregated according to their age, and they are placed in pens and fed a milk-replacement diet. These calves are usually raised out-of-doors and their pens undergo varying degrees of sanitation and clean-up. When trouble occurs, any calf from three days of age may be affected. Scours may be present or calves may be found dead, without any prior signs of illness. When received at the laboratory, we find a gaunt animal, the eyes sunken in the head, extremely dehydrated skin and subcutaneous tissue, and often the animal is dirty around the rear end and the tail. Autopsy reveals little or no lung involvement; epicardial and, at times, endocardial hemorrhage with an excessive amount of pericardial fluid; the stomach full of curdled milk and shavings or straw; and enlarged, juicy, mesenteric lymph nodes. Most of the time the intestines are empty of anything but bile-stained mucus, but at times we find a yellowish, diphtheritic cast in the intestine. There are varying degrees of intestinal mucosal reddening. Bacteriological cultures are made from the several internal organs, lymph nodes, and intestines, and either Escherichia coli or a Salmonella is usually recovered. Coliforms, except those recovered from the intestinal tract proper, are considered significant, but a Salmonella recovered from any tissue is considered significant. Sensitivity tests on the isolates are run and reported back to the veterinarian.

Disease control and prevention, under the above conditions, is almost an unsurmountable proposition. There is little that can be done to help this calf-raising industry, as it is economically unable to change its mode of operation. Time is of the essence as salmonellosis must be treated in the gastrointestinal tract to prevent losses. It is likely that these calves are infected at birth (some people believe "in utero"). Bacterins are of no benefit as the calf is too young to

produce immune antibodies. About all the veterinarian can do is try to have the owner get some oral, as well as parenteral, medication in the calf as soon as possible. Every animal is considered infected and is treated for several days. Sanitary measures include using smaller, segregated lots per pen, daily use of clean shavings or straw, and proper sterilization of feeding and watering equipment.

Environmental Factors

The significance of environmental factors is not always too clear. In the closed dairy herds, neither the source of infection nor the factors involved in spread of the infection are too clear. When the adult animals as a whole are affected clinically, there must be a rather severe exposure or a very debilitated herd, and the latter possibility is rather remote. The primary source of infection has not been discovered in this type of outbreak. Carrier animals, of course, may possibly be the source, and the virulence of the organism may be increased as it passes from animal to animal. The water trough or feed manger can become contaminated with infected fecal material and be excellent vehicles. S. typhimurium was isolated from the water trough in the mentioned outbreak north of Fresno, but S. dublin was not isolated from ditch water in the outbreak involving the certified milk dairy.

In feed-lot cattle, due to the stress factors of shipping and feed change, it would seem that carrier animals become clinical cases of salmonellosis. The stress factor of other disease conditions in feed-lot cattle can also debilitate them to such a degree that a Salmonella infection can take over. Cl. perfringens and Salmonellae, or coccidiosis and salmonellosis, are often found together in an individual animal in the feed lot and the paratyphoid would go unnoticed but for the primary disease.

In our calf-raising operations, the intrinsic factor of infection at birth from carrier animals cannot be overlooked nor eliminated. The environmental factors and sanitary principles that govern the spread of the infection from calf to calf are legion and have been mentioned previously. Correcting these adverse conditions is next to impossible.

Economic Factors

We are unable to estimate the economic significance of salmonellosis in California cattle. The extent of the problem is not known, for only a very small percentage of the losses are proven by laboratory examination. Many animals that are lost are not examined by professional persons. Once a diagnosis of Salmonella infection is arrived at for a ranch, the owner is prone to place all animals that die with similar signs in the same category and no more are examined. Possibly because of intensive medication, cases that arrive for laboratory examination may be found negative for Salmonella. All animals that are infected with Salmonella do not die, but they are stunted or retarded. We have not attempted to measure this economic loss.

Summary

Salmonella isolations from the bovine are on the increase in California. The three main types found are S. typhimurium, S. dublin, and S. newport. Salmonella infections in dairy herds are present, but are not a great problem. The problem is important in feed-lot cattle and is primarily a disease of imported feeders. Salmonella infections in our calf-rearing operations are widespread and difficult to control and prevent because of the type of husbandry practiced.

# RESEARCH NEEDS AND CONTROL PROGRAMS CONCERNING ANIMAL SALMONELLOSIS

By
H. Van Roekel*

Salmonella infections among animals were first detected at the close of the last century. Certain Salmonella types were detected as specific pathogens for certain animal and avian species and producing a rather definite syndrome and pathologic picture. Typical examples are Salmonella pullorum and S. gallinarum for the avian species, S. choleraesuis for swine, S. dublin for cattle, and S. abortus equi for horses.

Through the years the literature has become voluminous which reveals that many Salmonella serotypes have been described and that their distribution is global. More than 800 Salmonella serotypes have been isolated and classified into different groups on a biochemical and serological basis. A classification based on host susceptibility is not possible at the present time, although some types are rather restrictive in their host range. This is especially true for S. pullorum and S. gallinarum and has permitted the application of effective eradication measures to reduce the incidence to zero in some areas. More intensive eradication efforts should be made in all areas where these diseases are still a problem. In all foci or outbreaks of these diseases, the source of infection should be investigated, compulsory follow-up measures as to their eradication should be imposed, and relaxation in the testing programs for all breeding flocks should not be permitted for the moment.

Other Salmonella types play a prominent role in our domestic animals, including poultry. It has been reported that the avian species reveal a greater incidence of salmonellosis than do other domestic animals. However, the comparative incidence of Salmonella infections reported among animal species may not be a true one since more poultry specimens are submitted to the laboratory for bacteriologic examination, and also an appreciable number of Salmonella infections are detected in the routine testing of flocks for pullorum disease. It is very evident that more infected flocks and herds could be detected through some system of testing. However, at the present time a reliable and practical testing method for detecting infected flocks and herds is lacking. If an assessment is to be made of the incidence of these various Salmonella types, a reliable test should be developed. Numerous extensive surveys have been conducted concerning the presence of Salmonellae among poultry and eggs, processed foods, slaughtered swine and cattle, as well as other animals on farms. In the majority of these surveys a variety of serotypes were identified. It is noted that some serotypes appear more frequently than others. S. typhimurium is a striking example.

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There are other types less striking in their incidence. Some of these more frequent occurring types may have a high rate of infection among many hosts which may be significant as to their ability to attack, and survive in a multiplicity of hosts and to remain viable in the hosts' environment. This entire area of host-parasite relationship and environmental influence should be more extensively investigated concerning the more frequent occurring types to enable one to better understand the problem of their control and eradication.

In determining the presence of Salmonellae in animals, feeds, soil, litter, water, and inanimate objects, one must recognize the importance of the type of method selected in successfully isolating the organism. One may question how many diagnostic laboratories throughout the country employ reliable methods for the detection of these organisms. Federal publication entitled "Recommended Procedure for the Isolation of Salmonella Organisms from Animal Feeds and Meat Byproducts," ARS 91-36, October 1962, and other procedures reported in the literature should be helpful to isolate the various Salmonellae. This phase of the problem is equally as important as to have a standard serologic procedure to detect infections. Further recommendations could be made to improve the bacteriologic procedures for the isolations of the various Salmonellae.

Genus typing by a special phage for the Salmonella genus should be explored for application. Serotyping of the various isolated strains should be carried to its completion where it is deemed advisable. Also, phage typing should be developed more extensively, which will shed more light on the possible origin of the infection as well as establish possible relations between outbreaks of a specific Salmonella organism.

Pathogenicity studies of various strains within a certain serotype or for different serotypes should be undertaken. Only limited information on this aspect of the problem is available in the literature. Many reports are based on inadequate and poorly designed experiments.

Natural outbreaks of salmonellosis should be investigated from many different aspects; such as, infection rate, clinical manifestations, shedding of the organism, morbidity and mortality rates, serological response, survival of the organism in the litter, droppings, feed, water, the degree of egg-borne infection, influence on egg production, fertility, hatchability, and the occurrence of the disease in the progeny.

Environmental contamination with Salmonellae on farms, slaughtering plants, various types of equipment, supplies, personnel, insects, rodents, feral birds, and other animals should be tested or examined for the presence of Salmonellae. Contaminated premises should be checked periodically to determine how long infection may remain on the premises. Laboratory experiments could be designed to determine the resistance of the more prevalent types to environmental influence.

The resistance of these organisms to physical and chemical agents should be further explored, which may have a direct bearing on processing of animal products, as well as conditions on the farm and elsewhere. Is the pasteurization of whole egg products and possibly other products to be established? Other methods of sterilization or decontamination of Salmonellae should be considered. This is especially important when meat inspection programs are to provide wholesome products for human consumption. As veterinarians, we should recognize this and not have the medical profession and public health workers point out the weaknesses in our programs.

Any control or eradication program designed for Salmonella infections must take into consideration the source of the stock, source of feed, litter, water, and any other supplies -- all of which should be free of Salmonellae. Such conditions are difficult to establish with assurance. At the moment, the feed seems to offer the greatest obstacle. Ways and means should be provided so that Salmonellae-free feed can be obtained with certainty. Once this has been accomplished, one can move forward to providing an environment free of Salmonellae. This environment will require sanitary precautions that will not permit feral birds, rodents, vermin, insects, or any traffic that may introduce Salmonella infections.

In conclusion, it may be stated that salmonellosis constitutes a most complex and difficult problem that is of concern to human and animal health. Many aspects of this problem need further investigation in order to cope with the foci and outbreaks that may involve the various serotypes. We must expand interests and increase facilities in our animal disease diagnostic laboratories, which can be improved through professional workshops in the various States. Also, it is recommended that some person or persons in each State be delegated with the responsibility to coordinate the efforts concerned with this disease problem and cooperate with the Federal Government to formulate a national surveillance program on salmonellosis.

Occurrence of Some Salmonella Types
Among Certain Animal and Avian Species*
January 1957 - July 1961

| Type | Turkeys | Chickens | Ducks | Swine | Goats & Sheep | Cattle | Horses | Dogs |
|---|---|---|---|---|---|---|---|---|
| anatum | 176 | 59 | 1 | 8 | - | 26 | 3 | 1 |
| blockley | 16 | 82 | - | 4 | - | - | - | - |
| chester | 106 | 52 | - | - | - | - | - | - |
| choleraesuis var. kunzendorf | - | 12 | - | 353 | - | 4 | - | 1 |
| derby | 52 | 17 | 1 | 27 | 2 | 1 | - | 2 |
| dublin | - | 7 | - | - | 1 | 50 | 2 | 2 |
| enteritidis | 63 | 25 | - | - | - | 50 | 99 | - |
| heidelberg | 159 | 127 | - | 2 | - | 2 | - | 1 |
| infantis | 78 | 94 | - | 2 | 1 | 1 | 3 | 2 |
| muenchen | 95 | 16 | - | 1 | - | 1 | - | - |
| newport | 165 | 6 | 1 | 3 | 3 | 57 | 3 | 4 |
| saint paul | 114 | 18 | - | - | - | - | 2 | - |
| san diego | 215 | 10 | - | - | - | 2 | - | - |
| typhimurium | 612 | 282 | 9 | 45 | 11 | 185 | 49 | 40 |

*Source -
Moran, A. B., Proceedings of U. S. Livestock Sanitary Association, 1961.

# INTERSPECIES TRANSMISSION OF SALMONELLA

By
A. I. Flowers*

The first isolation of an organism of the genus Salmonella from an animal source was Salmonella choleraesuis from swine by Salmon and Smith (24) 1/ in 1889. In 1895, Moore (18) isolated a paratyphoid from an outbreak of infectious enteritis in pigeons. The first report of paratyphoid in turkey poults in the United States was that of Rettger, et al., (23) in 1933, although Pomeroy and Fenstermacher (22) had observed the infection in turkeys in Minnesota in 1932. As the importance of the disease began to be recognized and received increasing attention, it was soon realized that salmonellosis was a problem in virtually all species throughout the world, including man. It is virtually impossible at present to compile valid statistics of the incidence of Salmonella infection in man or any species of domestic animal because of inaccuracies in diagnosing and reporting outbreaks. In man and domestic animals, infection often produces clinical signs only in the young, with many apparently healthy adult carriers remaining undetected. Also, many salmonellosis outbreaks in man involve only one or a small number of individuals and are not recognized. Conversely, most so-called staphylococcic "food-poisoning" outbreaks involve considerable numbers of people and thus are more likely to be recorded, so that comparative statistics are misleading.

## Reservoirs of Infection Common to Several Species

In the past, it has been customary to refer to host-adapted types or so-called "primary salmonellosis" of different species as compared to nonhost-adapted types. Hinshaw and McNeil (12, 13) and Edwards (4) have indicated that many types occur both in animals and man. Edwards (4) pointed out "that there is a distinct correlation between the presence of the organisms in lower animals and in the human population." Obviously a high incidence of salmonellosis in any particular species of animal represents a potential reservoir of infection to other species to a degree directly related to the level of association of the two species.

Fowl. The tremendous population of domestic poultry (about 600 million chickens on U. S. Farms on January 1, 1957, according to estimates of the U.S. Department of Agriculture) together with the high incidence of infection, necessitates their consideration as the greatest single reservoir of Salmonella (4, 8). According to the report of Perelli-Minetti, et al., (20) the incidence of salmonellosis in turkeys presented for autopsy in California in 1947-1948 amounted to 41.4 percent. In Texas, in 1958-1959, of 136,000

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1/ Underscored numbers in parentheses refer to Literature Cited at end of paper.

turkeys tested at the College Station Laboratory there were 356 reactors submitted for bacteriological examination with S. gallinarum being isolated from one flock and paratyphoid organisms from 13 flocks (3.6%). It should be emphasized that neither the incidence reported from California nor that from Texas represents the true picture since the first sample was of birds suspected of being ill while the latter was from birds reared under a Salmonella control program. Transovarian transmission may occur but it appears that in most instances fecal contamination of the shell allows for penetration of the organism through the pores prior to or during incubation. It is interesting to note that the prevalence of serotypes of Salmonella varies with the locality. S. typhimurium is the serotype most frequently isolated from poultry in most sections of the United States but in 1962-1963 in Texas this serotype was identified in only 4 of 30 isolations. The incidence of salmonellosis in turkeys in Texas is increasing.

Swine. Although swine are considered to be the reservoir for S. choleraesuis this species has also been recovered from man, cattle, dogs, chickens, turkeys, foxes, and canaries (1). However, Bruner and Moran (3) found that of 1,056 outbreaks of salmonellosis in swine, 810 of the isolations were S. choleraesuis. Galton, et al., (10) reported that isolations were much more frequent from swine in abattoirs than from animals on the farm and pointed out the importance of the sales yard, holding yard, etc., in the spread of the organisms. Moore (17) reported the isolation of 15 as yet untyped paratyphoids from the feces of 171 apparently healthy pigs on feeding trials in 1961 at the Texas Agricultural Experiment Station. One outbreak in an SPF (Specific Pathogen Free) herd appears to have been transmitted via feed products but this has not been substantiated.

Rodents. S. typhimurium and S. enteritidis are frequently isolated from rats and mice but these animals usually harbor any of the other serotypes common to their environment. This is substantiated by a recent report in which Pigford, et al., (21) found a high percentage of S. typhimurium in isolations from rats trapped in or near restaurants, with a direct correlation to serotypes isolated from surfaces and residues in kitchen areas of those restaurants.

Ruminants. S. dublin and S. typhimurium along with other serotypes are frequently encountered in cattle and sheep. Murdock and Gordon (19) reported the isolation of S. dublin from the feces of 24.5 percent of apparently healthy cattle in Northern Ireland. Salmonellosis has also been reported as a disease problem in cattle in other parts of the world and is often transmitted to man.

Household Pets. Many species of birds, small rodents kept as household pets, and dogs and cats serve as reservoirs of salmonellosis. As early as 1938, Magnusson (15) reported the isolation of paratyphoid organisms from dogs. Since that time there have been many reports confirming the incidence of Salmonella in this species. Jungerman and Grumbles (14) reported the isolation of nine different serotypes from fecal swabs from 100 apparently

healthy mature dogs. Young puppies, as in other species, often develop a gastroenteritis following infection with Salmonella but in many adult dogs a "transient" carrier state exists.

Reptiles, Fish, and Insects. Snakes as carriers of Salmonella was reported by McNeil and Hinshaw (16) in 1944. There have been many reports since that time concerning the incidence of Salmonella in reptiles, fish, and amphibia. Flies, ticks, and other insects sometimes harbor the organism but it is not known whether this is merely a reflection of their environment or not.

Food and Feed Ingredients. Erwin (6) in 1955 reported the isolation of Salmonellae from 3 of 203 samples of poultry feed. In 1956, S. panama and S. newport were isolated by personnel of the poultry disease diagnostic laboratory at College Station, Texas, from one sample of poultry byproducts reported to cause gastroenteritis in dogs when incorporated in finished dog meal. Since that time there have been many reports in which the incidence of Salmonella isolations from animal feeds ranged from 18 to 24 percent reported by Watkins, et al., (25), the percentage depending on the type feed or feed ingredient being tested. Many finished feeds, especially pelletized products and canned foods appear to have a very low rate of contamination or none at all. The report of Galton, et al., (9) of the isolation of organisms from 23 percent of samples of fresh sausage and from 12.5 percent of smoked sausage emphasizes the magnitude of the role of foods of animal origin as sources of infection. There are also numerous reports concerning the incidence of Salmonellae in eggs and egg products.

Man. As pointed out in the report of Edwards, et al., (5) humans have long been known as frequent carriers of Salmonellae. Different reports estimate the carrier rate of range from 0.2 percent in the general public to no less than 63 percent among food handlers.

## Mode of Transmission

It is the opinion of the author that the importance of animal feeds as a source of Salmonella infection for the various species of domestic animals has not been fully appreciated. The conclusion of various investigators that there is a direct correlation between the serotypes isolated from human and lower animals within a given locality appears to be substantiated by the statistics, and this concept may apparently be extended to include food and feed ingredients. The most common mode of transmission of the disease in all species of animals is direct or indirect contact.

In poultry, transovarian transmission is known to occur, but much more common is the penetration of the eggshell by organisms deposited there while the egg is in contact with the gastrointestinal tract. Transmission within the incubator or hatcher by contact or inhalation is frequent. The disease is further spread by contact or ingestion of feces or fecal contaminated water or feed during the growing period.

Swine apparently transmit the disease from apparently healthy carriers to others of the same species, and to other species with which they come in contact, including man, as evidenced by the report of Galton, et al., (10).

Rodents such as rats and mice are numerous where sanitary conditions are minimal and particularly where the food supply is abundant such as slums and food-processing or feed-storage areas. Transmission again is by direct contact and ingestion of fecal contaminated food or water. Ample opportunity is provided for infected individuals to contaminate subsequent lots of human or animal food so that the environment remains constantly contaminated.

Transmission from ruminants is primarily by direct contact and contaminated water supplies. Humans become infected by consumption of milk from infected animals, and in slaughter and packing houses by contact with contaminated tissues.

It seems probable that household pets transmit salmonellosis to their owners and vice versa because of constant and intimate contact. Again, many household pets no doubt receive their initial exposure from contaminated food.

Salmonellosis in fish originates from human contamination of the water in which they live. Reptiles, no doubt, are most frequently infected by eating small rodents and birds that are harboring the infection.

It thus seems apparent to the author that with the obvious interspecies transmission of Salmonellae, preventing the introduction into animals that serve as a source of food for man is the obvious answer to the problem. All species of animals and offal are utilized as protein sources for poultry and livestock feeds. Contamination of these materials leads to contamination of the finished products and subsequent introduction of the infection to domestic animals and man. Boyer, et al., (2) demonstrated rather conclusively that turkey poults became infected from contaminated feed. A similar case involving S. derby in baby chicks and feed was investigated by the author. Unpublished data on feeding trials at the Texas Agricultural Experiment Station have demonstrated that as few as 1000 organisms per gram of feed will produce detectable serological titers and infection in some individual turkeys.

Food handlers and animal caretakers become infected in due course, often remaining carriers and transmitting the infection to other humans or domestic livestock. Rodents and lower animals found in great numbers in population centers perpetuate the infection.

Humans have perhaps not received the attention they deserve as reservoirs of infection. The role of food handlers has been mentioned but, in addition, the disease has been transmitted from person to person as mentioned by Edwards (4) and others.

## Value of Serological Diagnosis and Efficacy of Antigens

Serological testing and eradication programs for salmonellosis in fowl are well advanced and reasonably successful, particularly with regard to turkeys and chickens. These types of tests are used in control programs in Texas, i.e., whole blood rapid plate test, serum rapid plate test, and the tube agglutination test. The latter is the most reliable and is the only official test for turkeys in Texas. Most flocks are tested using S. pullorum antigen for the detection of S. pullorum and S. gallinarum. Because of antigenic components in common (somatic antigen 12), pullorum antigen will often detect paratyphoid belonging to group B, although the titer is not as high as with the homologous antigen. In addition several States, including Texas, offer a voluntary paratyphoid testing program using S. typhimurium as the test antigen. Several investigators are attempting to develop a paratyphoid antigen that would be inclusive of most if not all paratyphoid serotypes, and some progress has been made. Such experimental antigens are not used to any extent at present.

The same tests mentioned above may be used for other species of domestic poultry such as ducks and geese, but some difficulty in interpretation is encountered. Goose sera is cloudy due to the high fat content, making reading of results difficult, but in the author's experience the results are reasonably reliable.

Serological testing in cattle is somewhat different than that of poultry and results are not as reliable. According to Henning (11) and Field (7) cattle seldom have significant titers to the "O" antigen, but titers to the flagellar antigen in excess of 1:160 or 1:200 are significant. In many instances, however, test results did not correlate with cultural examinations, with both positive and negative discrepancies.

## Summary

Salmonellosis is an important worldwide problem involving all species of wild and domestic animals and fowl. With few exceptions, serotypes are not host specific, and cross infection is frequent. Many carriers exist in most species including man. Transmission is most often due to ingestion of fecal contaminated food or water but infection by direct contact does occur. Control by sanitation, rodent control, and protection against contamination of feeds appears to the author to be the answer to the problem. Improved serological methods of detecting carriers are badly needed.

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# EPIDEMIOLOGY OF SALMONELLOSIS

By
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The success of any control program against salmonellosis depends on the extent that "shoe leather" epidemiology is used.

In every outbreak of salmonellosis it will be necessary for the epidemiologist to investigate every species of animal life from the invertebrate to man to determine the reservoir source of infection. This will be illustrated by the following:

1. Our attention was first called to the possibility of snakes acting as another reservoir of Salmonella, when Salmonella meleagridis was isolated from a gopher snake caught on a ranch where this Salmonella type was causing losses in turkey poults. The ranch where this snake was caught is located on the Mohave Desert. The owner has experienced severe losses from salmonellosis in his turkey poults over a period of several years, and a total of seven types have now been isolated from turkeys, chickens, and snakes from this ranch. Since the owner has his own hatchery; maintains his own breeding flocks; and does not introduce any outside stock, it has been difficult to explain the origin of the numerous Salmonella isolated.

A total of 25 snakes were caught on this ranch and seven of them yielded Salmonella. The list of snakes from this ranch included 21 gopher snakes, 3 garter snakes, and 1 red racer. Rattlesnakes are found in that area, but none was killed on the ranch during the survey. During 1944, the owner carried out an intensive snake-eradication campaign, and it is of interest that for the first time in four years, no Salmonella reactors were found in his turkey breeders when they were tested in the fall of 1944.

Three snakes that yielded Salmonella came from two turkey ranches where unexplained outbreaks of salmonellosis had occurred. In each case, the types isolated were the same ones that had caused losses in turkeys on the ranches. S. rubislaw, isolated from another infected snake, has not caused losses on the ranch where the isolation was made, but it has been known to cause mortality in turkeys elsewhere. The types isolated from snakes have all been the cause of food poisoning outbreaks in man, and reptiles have now been added to the list of animal reservoirs for Salmonella.

A total of 15 snakes were autopsied for the San Diego Zoo. Seven of the 15 were rattlesnakes. Paracolon type 10 was isolated from at least 4 of these.

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There are several facts that show the relation of the isolations from these snakes to paracolon type 10 infection of turkeys. This cage was always kept as an exhibit of snakes caught in San Diego County or adjoining territory. This is the only cage that suffered epidemic losses, and the only one from which this paracolon type was recovered. Losses were caused by this type in turkey poults in the area, and rattlesnakes were present on at least two of the turkey ranches from which this type was isolated.

A new Salmonella type was isolated from the intestine of a female green mamba snake which was submitted to us for autopsy by the San Diego Zoological Society. It was one of a pair purchased a few weeks previously for exhibition. The native habitat of the snake is western Africa from the Senegal to the Niger. Since the specimens had so recently been imported, the type name senegal was selected for this Salmonella. This snake died suddenly and had not been ill at any time following its arrival at the zoo. On autopsy it was noted to be poor in flesh, but not emaciated. The posterior intestine was studded with ulcers. Except for a slight congestion of the liver and mucosa of the stomach no other abnormalities were noted.

2. On one ranch an unexplained outbreak of S. typhimurium infection caused severe mortality in a brood of young poults that were hatched from eggs laid by breeders that were negative to both H and O types of S. typhimurium agglutination tests at the beginning of the 1944 hatching season. A retest of the flock after the outbreak occurred revealed 3.7 percent reactors. In a survey to determine the possible animal reservoirs that might account for the transmission to the breeders and subsequently to the poults, 2 quail, a crow, a garter snake, a king snake, 2 gopher snakes, 3 adult cats and 4 young kittens have been examined to date. S. typhimurium was isolated from 2 of the adult cats and from one of the gopher snakes. One of the cats yielded the organism from the lungs, mesenteric lymph nodes, and intestine, but it was only isolated from the intestine of the other. The heart blood, liver, lungs, kidneys, ovary and intestine of the snake all yielded S. typhimurium. Both of the infected cats were known to be frequent visitors to the turkey yards. Egg shells and feathers in the intestine of one, and feathers in the intestine of the other furnished further evidence of these visits. The snake was caught near the yard where the survivors of the outbreak were penned.

On the second ranch, S. typhimurium infection has been a problem for several years, and each year outbreaks have occurred that could not be traced to turkey carriers. This spring the owners were asked to submit poults frequently to the laboratory from each hatch regardless of how little mortality occurred. Poults submitted from 2 lots at 2 and 10 days of age were negative for all Salmonellae. A week later a garter snake was caught in one of the brooder yards, and sent alive to the laboratory. From its liver, spleen, testes, and intestine, S. typhimurium was isolated. About this same time several poults appeared abnormal, and two days later poults from both lots submitted to the laboratory for examination yielded S. typhimurium.

Six weeks later 320 house flies were caught on "sticky" fly paper strips hung in two brooder houses on the second ranch. These were divided into 8 lots of 40 each and put into tubes of tetrathionate broth. From 4 of the 8

lots, S. typhimurium was isolated. It is of interest that the 4 positive isolations came from the brooder house used for the survivors of the outbreak mentioned above.

3. S. chandans, a new type, was isolated from the intestine of a Pacific fence lizard, and S. bonarensis was isolated from the intestine of the same lizard.

The isolation of S. rubislaw is reported from 3 or 12 Pacific fence lizards examined. From the same group of lizards, two sucrose-positive paracolons which have Salmonella antigenic components were also isolated.

4. S. montevideo was first isolated from an outbreak in 10-day-old poults in May 1942. Sand recently removed from a cesspool of a ranch tenant house was used by mistake as a source of grit for these poults 3 days before the losses started. Deaths occurred only in the brooders where this sand was used. This suggests the possible transmission of this type from human beings to poults. The same type was later isolated from a sump under the drinking fountain in the yard used for the survivors of this outbreak. One outbreak on another ranch has been traced to eggs laid by these survivors. A human case of gastroenteritis caused by S. montevideo resulted from handling the poults in this outbreak.

SUGGESTED READING

Edwards, P. R.
1958. Salmonellosis Observations on Incidence and Control. Animal Disease and Human Health. Annals of the New York Academn of Science. v. 70, article 3.

Proceedings of the U.S. Public Health Conference
1963. Epidemiology of Salmonella.

# LABORATORY SUPPORT AND REPORTING OF SALMONELLOSIS

By
Morris S. Cover*

The basic geographic unit of a Salmonella control program is the official State diagnostic laboratory. Certainly this is the basic unit either for making a diagnosis or confirming one. The veterinarian and/or poultry pathologist is the front line unit - for here the trouble is first revealed. The farmer, owner, or grower, calls for scientific and professional assistance because of a disease problem on the farm. The average, intelligent professional practitioner (also diagnostician) will be able to make a tentative on-the-spot diagnosis and recommend an individual control program for that farm or case. But, he will want confirmation of his diagnosis; thus, the State or area diagnostic laboratory enters the picture. In each case, this laboratory should have (and usually does have) a routine procedure for confirming a Salmonella diagnosis. Thus, it appears that at the State or area level, there rests considerable responsibility for the control of this or any other disease.

In my State - Delaware - as in almost all others, there are several diagnostic laboratories. Their existence may stem from one or more varied reasons.

1. They may be administered by the State Board of Agriculture and thus, are essentially regulatory in nature.

2. They may be established by commodity groups and are therefore characteristically service organizations.

3. They may be a part of a commercial venture and are therefore established as a sales promotion feature. This includes three types - those that sell medicaments and biologics, those that sell poultry or animals, and those that sell equipment.

4. They may be formulated as a part of a university, college or agricultural experiment station program and are therefore research oriented.

In no way do I wish to rate a diagnostic laboratory because of its genesis--certainly any of the above types of organizations can do an excellent job in making a diagnosis. The accuracy of their diagnostic service is dependent largely on the professional ability and training of the personnel. However, there is some difference in each case of the obligation in regard to reporting. This is not to say that a moral obligation does not exist in all

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cases to report to proper authorities in order to confine and/or reduce the disease in an area, State, or the entire Nation. I am pointing out that there are differences of parental pressures in each case, but this situation emphasizes the basic nature of the official State or area diagnostic laboratory.

One specific and important point I do wish to emphasize about State and area diagnostic laboratories is that all diagnosticians should be aware of the diagnostic facilities available to them within the area. Many laboratories do not have the time and/or equipment to make a complete and thorough investigation into difficult cases. All laboratories, therefore, should know where to send specimens for a quick and accurate diagnosis. Also, in my State, we have frequent meetings of all diagnostic personnel from all laboratories within the State to discuss the following items.

1. Methods of making a diagnosis and most important, the interpretation of the results.

2. The appearance of new diseases.

3. The possible outbreak or increase in a serious disease within the area.

4. Exchange of information concerning the epidemiology of diseases.

I cannot overemphasize the invaluable information and cooperation obtained from our interstate - area meetings. Of course, meetings would be difficult in larger or less populated areas, but still the expense and time spent would be well worthwhile.

From the State or area level, the next step in confirmation is at the national level. This is the responsibility of the Federal Government. I am indicating here that in those cases where the conditions warrant it and as many cases as possible, an exacting and complete identification should be made on the etiologic agent. In such cases, the organism would have been already identified as a Salmonella. All serotyping should be done by a Federal laboratory or laboratories. Such work would be done by the U.S.D.A. National Animal Disease Laboratory, Ames, Iowa.

The final phase of the reporting is the retrograde activity. Once the diagnosis is made, the official State laboratories should check with the regional diagnostician of the case in question. Not all cases can, nor should be followed out completely, but as many as possible should receive some attention. This may mean that a phone call or letter to the original diagnostician may suffice in some cases; while a complete personal investigation would be necessary in others.

As indicated previously, the accuracy here is dependent primarily upon the professional ability and training of the personnel. Also important, is the efficiency of the laboratory as influenced by its work load. Does the laboratory have time to do the complete job necessary to make a diagnosis? The techniques used by the confirming diagnostician are important.

The exact procedure to be used by this laboratory will be governed by the national requirements. These instructions are as follows:

"Subpart B - Bacteriological Examination Procedure

Sec. 147.11 - Laboratory procedure recommended for the bacteriological examination of reactors. (a) The pericardial sac, peritoneum, oviduct and any visibly pathological tissues should be cultured on beef extract agar or tryptose agar by means of sterile swabs. Sterile technique should be followed. (Primary culture of these organs in a suitable nutrient broth and transfer to a suitable nutrient agar is optional.)

(b) The following organs should be aseptically collected for culture:

(1) Heart (apex, pericardial sac, and contents if present.);

(2) Liver (portions exhibiting lesions or in grossly normal organs the drained gall bladder and adjacent liver tissues.);

(3) Ovary-Testes (entire inactive ovary or testes but if ovary is active use own judgment and include any atypical ova.);

(4) Oviduct (if active include any debris and dehydrated ova.);

(5) Pancreas; and

(6) Spleen.

(c) A composite sample of the organs listed in section 147.11(b) should be ground in a sterile mortar or suitable blender. Individual organs may be used if desired. Nutrient broth should be added as a diluent. Ten cc. of this suspension should be inoculated into 100 cc. of either Selenite F broth or Tetrathionate broth and into 100 cc. of a suitable noninhibitory nutrient broth.

(d) After 24 hours incubation at 37° C. a loopful of the broth cultures from each flask should be streaked on a suitable noninhibitory solid medium such as tryptose agar and one of the following selective media: Salmonella-Shigella (SS), MacConkey, Brillant Green, Bismuth Sulfite, or Desoxycholate Citrate Lactose Sucrose (D.C.L.S.) agar. (All of these media may be obtained in dehydrated form.) If no suspicious colonies are observed after 24 hours incubation the enrichment broths should be restreaked on solid media.

(e) A portion of the crop wall and intestine to include the cecal tonsils are put into either Selenite F or Tetrathionate broth and incubated for 24 hours at 37° C. Transfers should be made from the broth onto agar plates as indicated in section 147.11(d).

(f) Suspicious single colonies should be subcultured on nutrient agar or triple sugar iron agar slants and incubated for 24 hours at 37° C.

(g) Cultures should be transferred to the following fermentable media for identification: dextrose, lactose, sucrose (saccharose), mannite (mannitol), maltose, dulcite (dulcitol) and salicin broths. Suitable tests also should be conducted for the detection of indol, hydrogen sulfide, acetylmethylcarbinol, and urease production. Motility or nonmotility is demonstrated by inoculation of a suitable semisolid medium. For the Gram stain a 24 hour nutrient agar slant culture should be used.

(h) All Salmonella cultures isolated should be serologically typed."

I would like to refer to an article published some tine ago by Jungherr, et al., on Techniques for the Bacteriologic Examination of Reactors to Pullorum Disease Antigen. This was published in the 1950 Proceedings of the American Veterinary Medical Association Annual Meeting. It presented a comparison of successful isolations from various body areas. A summarization is given here:

| | | Positive isolations |
|---|---|---|
| Bone Marrow | 1% | |
| Gall Bladder | 52 | (2) |
| Heart-Blood | 6 | |
| Intestine | 21 | (5) |
| Liver | 1 | |
| Ovary | 71 | (1) |
| Oviduct | 26 | (4) |
| Peritoneum | 35 | (3) |
| Pericardial Sac | 15 | (6) |
| Spleen | 6 | |
| Testes | 50 | (1) |

Summary:

| | |
|---|---|
| Gonads | 71% |
| Gall Bladder | 52% |
| Peritoneum | 35% |
| Oviduct | 26% |
| Intestine | 21% |
| Pericardial Sac | 15% |

An evaluation of the enrichment media was as follows from 81 cases.

| | Tetrathionate | Selenite |
|---|---|---|
| Gall Bladder | 16 | 24 |
| Intestine | 9 | 18 |
| Ovary | 20 | 33 |
| Total | 45 | 75 |

Their report on selective media was as follows: (after selenite)

| | SS | MacConkey | Bismuth Sulfite |
|---|---|---|---|
| Gall Bladder | 21 | 22 | 24 |
| Intestine | 15 | 8 | 16 |
| Ovary | 29 | 25 | 32 |
| Total | 65 | 55 | 72 |

Their recommendations were as follows:

"A Minimum Standard for the Bacteriologic Examination of Pullorum Reactors

"1) Pericardial sac, peritoneum, and oviduct are cultured on beef extract agar slants by means of sterile cotton swabs. Sterile cotton swabs are suggested because they can be kept readily available and also lend themselves to culturing serous or mucous surfaces. Inoculation needles may not pick up sufficient material, especially when hot. Sterile technique should be observed. (Primary culture of these organs in beef extract broth and transfer to beef extract agar is optional.)

"2) Ovary or several small, particularly malformed, ovarian follicles, gall bladder, ileal contents, and portions of pathologic objects such as cysts, if present (approximately 1 g. of material per 20 ml. of enrichment medium in 1-in. tubes), are placed into individual tubes of selenite broth. After twenty-four hours of incubation at 37° C., a loopful of culture from each tube is streaked on 1/3 plate of SS agar and bismuth sulfite agar. (Tetrathionate broth with or without 1:200,000 brilliant green, MacConkey's, Desoxycholate, and D.C.L.S. agar are optional.)

"3) Suspicious single colonies from agar slants and plates are subcultured on agar slants. Cells from the subcultures are transferred to tubes on nutrient carbohydrate broth.

"Inoculation into 1 percent tryptone broth for exclusion of indol production and into urea medium (Proc. Soc. Exptl. Biol. & Med., 47, (1941): 108) for exclusion of urease activity is advised.

"Nonmotility should be demonstrated in broth or 0.3 percent semisolid agar cultures. A 24-hour growth of beef extract agar slants should be used for gram stains and, if desired, for slide agglutination tests with known S. pullorum antiserums, preferably both S and X types.

"Choice of Organs to Culture

"Of the ten organs listed in the table (counting both gonads as one organ), it will be seen that the ovary, gall bladder, peritoneum, oviduct, intestine, and pericardial sac, in this order, gave the highest percentage of successful isolations. The much less frequent isolations from heart blood, spleen, liver, and bone marrow were never made from these organs alone

"The gall bladder, intestine, and ovary from 30 chronic and 11 acute cases were cultured comparatively in tetrathionate with brilliant green, and in selenite broth, respectively. With a single exception, selenite yielded a higher number of successful isolations on follow-up medium than tetrathionate, the aggregate difference amounting to an increase of 30 over 45 (66%). Aside from the statistical evidence, simplicity of selenite preparation favored its adoption.

"Choice of Selective Mediums

"In the above comparative study, both tetrathionate and selenite broth cultures were subcultured on SS, MacConkey's and bismuth sulfite agar. For tabular purposes, the data from the preceding selenite broth experiment were utilized. It will be seen that, by and large, bismuth sulfite and SS agar permitted the highest number of specific isolations. It also seems that these statistically favored, selective mediums tend to suppress growth of coliform organisms."

There is no doubt that a national reporting plan as a part of an increased national effort will be necessary for eradication of this disease. In my opinion, this can only be done by making the official State laboratory the key organization in this effort. To me, it will certainly fail if the reports are sent directly to a Federal group and summarization sent out from here. If in some way we can stimulate local interest in this reporting and keep it in local color, success is a greater possibility. This does not preclude that the Federal authorities cannot collect and organize the statistics on a national level. If the U.S. Department of Agriculture representative can become an integral part of the State or area system, then a national reporting system can succeed.

# SALMONELLOSIS EPIDEMIOLOGY IN WISCONSIN

By
A. I. Moyle*

During July and August 1963, licensed rendering plants in Wisconsin were surveyed to determine those factors affecting the bacterial quality of the finished products. Approximately one day was spent studying the operations of each plant. In addition to a sanitary inspection, swabs were taken of equipment, petri dishes were exposed, and samples of the finished products were collected.

All samples for bacteriological examination were forwarded to the Wisconsin Animal Health Laboratories.

The coliform count has been used as an index of general sanitary conditions in the plants.

When Salmonellae were isolated, they were group serotyped and referred to the National Animal Disease Laboratory for speciation.

There was no apparent correlation between plant sanitation and the presence of Salmonella in the finished products. Duration and type of storage seemed to influence bacterial population. Handling methods and opportunity for contamination by animals, birds, or arthropods may also be significant.

Representative plants were selected for further study to ascertain what progress could be made toward production of a Salmonella-free product.

Small, medium, and large plants were chosen that handled both dead animals and slaughterhouse offal. Another plant was selected for special study because it handles a large volume of poultry offal and infertile eggs. This plant markets much of its meat scrap directly to poultry producers.

Efforts have been made to obtain monthly samples from the four plants to check seasonal variations in the degree of contamination and increase the probability of discovering variations in the degree of contamination caused by infective raw materials.

The survey has emphasized need for more basic research on the role of Salmonella in contamination or recontamination of animal byproducts. Some of the questions which must be answered are:

1. Degree of contamination -- is there a tolerance level?
2. Effect of storage -- do Salmonella multiply, remain dormant, or die during storage?

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3. Relative importance of contamination and recontamination -- are the Salmonella in rendering plant products the result of contamination by rodents, wild birds, pets, and arthropods, or is the primary source improper handling of raw material?

4. Pathogenicity of Salmonella species -- a better understanding of their role as a cause of disease.

5. The possible role of epizootics as a source of Salmonella in raw materials and how this may affect the bacterial population of finished products.

As a result of this survey, future study will be directed as follows:

1. Check other areas of possible contamination between the rendering plant and the consumer; i.e., wholesalers, mixers, feed companies, haulers, and on-the-farm storage.

2. Follow dead animals from known outbreaks of Salmonella infection through a rendering plant and observe possible impact on the finished product.

3. Examine apparently healthy animals to determine the frequency of infection by Salmonella.

4. Controlled experiments with stored feeds to observe the effect of storage on viability and virulence of Salmonella.

5. Methods that may be used to sterilize feed.

Summary

In a survey of Wisconsin rendering plants little correlation was found between sanitary practices and the incidence of Salmonella in animal byproducts produced. Duration and conditions of storage seemed most significant.

There is need for more basic research on Salmonella.

Further epidemiological studies are suggested.

Attachment:
Rendering Plant Operation

Rendering Plant Operation
Factors Affecting Recontamination of Product

As the result of an extensive survey of rendering plant operations in the State of Wisconsin carried out during July and August of 1963, the following factors were chosen as the most important ones which might have a bearing on the problem of recontamination of meat scrap produced in these plants to be used in animal feeds:

1. Kind of Material Rendered.--Percent of packing house offal and percent of dead animal pickups. It is felt that where the whole animals are picked up and brought in there is more opportunity to bring contaminants into the plant and the need for greater care and sanitation in the plant operation is imperative to keep this source of contamination under control.

2. Preparation of Material.--This refers primarily to the use of a chipper or its absence. Material that is macerated before cooking is easier to cook and lends itself to later processes more readily. There is no problem with large bones, which accumulate and become contaminated before being ground and blended into the finished product.

3. Cooking Process.--Whether material is cooked under pressure or not is important more for the condition it leaves the cooked material in than for the degree to which it is cooked. For instance, a plant using pressure within the cooker could get by without a chipper because the pressure softens the bone material and helps in later processing.

4. Grease Removal.--Two methods used, the expellor and the press, are compared and the expellor is preferred for our purpose because it lends itself readily to automation, and it is felt the least amount of handling of the finished product is the most desirable.

5. Handling of Material.--As stated above, the less the finished meat scrap is handled in the rendering plant environment the better. Conveyor systems seem to be adaptable in many plants and should be made more use of.

6. Storage Place and Methods.--This again ties into the amount of handling the finished product gets, but more important here is the degree of exposure it gets to contamination from raw materials while in storage.

7. Grinding and Bagging.--This is one more operation that requires handling and if care is not exercised may expose the material to contamination.

8. Mixing Operations.--Plants vary greatly in the number of operations their material is put through before it is put on the market. Some do extensive mixing and blending operations in the rendering plant atmosphere, which may provide opportunity for more contaminants to get into their product.

The above eight items have to do with the material rendered out and how it is handled within the plant. The remaining items have to do with the general plant operation and sanitation and how they might have a bearing on recontamination of the meat scrap.

9. Plant Organization.--This is particularly important with reference to the situation of the clean and dirty areas within the plant in relation to each other and to the storage areas.

10. Plant Sanitation.--The need for this should be obvious in a rendering plant situation. Unfortunately the surveyor found it grossly neglected in some areas.

11. Water Supply.-- The importance of an adequate water supply to rendering plant operation should be apparent. Cooking, cleaning, and vapor control all depend on the availability of an adequate supply of water.

12. Waste Disposal.--This is a major problem in a rendering plant operation, and requires good sewage disposal methods and adequate facilities for handling a large volume of waste materials.

13. Odor Control.--While not essential from the standpoint of recontamination it may still be considered a factor, as odors draw flies and other vermin as well as repel neighbors. Satisfactory odor control also aids in attracting better quality help in the plants.

14. Fly and Pest Control.--Constant war must be waged against flies, roaches, and rats and mice in rendering plants. Prime conditions are present for the propagation of these pests and only constant vigilance keeps them down. Contamination of the finished meat scrap from these sources is a major consideration.

Using the above fourteen items as a basis for comparison, the surveyor has graded the eighteen operating rendering plants in the State and has come up with the following general classification:

Six plants rated above average.

Two plants rated average.

Ten plants rated below average.

In addition to these general items of comparison nearly all plants have special problems that are unique to their plant organization, operation, or management and that tend to introduce variable factors into the picture and affect the accuracy of any comparison based on the above factors alone.

# DISCUSSION AND WORKSHOP ASSIGNMENTS

By

John W. Walker*

At the conclusion of the formal presentation, I again express appreciation to our distinguished guest speakers and commend you on these excellent talks. But even more, we appreciate the vast experience and many years of devoted service you have given in the field of animal disease control and research. We recognize this and are happy that you are willing to share this knowledge with us.

Attached to your program is the workshop assignments. We are asking the chairman of each group to take his people to the designated conference room and consider the points mentioned on this sheet. Please do not limit your thinking to these points, these are the problems we recognize. Consider those you feel are the most essential in the formulating of Salmonella control activities.

At the end of the workshop period, please bring your written reports back to this group to be read and discussed. It is hoped that recommendations from the workshops will be listed in order of priority to help the Division chart its course of action in the most efficient manner.

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## RECOMMENDATIONS OF THE WORKSHOP ON LABORATORY SUPPORT AND REPORTING

The Workshop recommends that each State or area provide a diagnostic laboratory that will be able to confirm field diagnoses of Salmonella and Arizona species, make bacteriological examination of feed and feed ingredients, and isolations from feral birds, farm rodents, and other live carriers. This laboratory should be able to isolate and bacteriologically characterize, including the biochemical tests, the Salmonella and Arizona species. This should be a State administered and supported facility (in cooperation with the U.S. Department of Agriculture), which is in close contact and cooperation with the commercial, private, and other diagnostic laboratories within the State.

All serotyping shall be done by central laboratory(ies) under the administration and by the support of the U.S. Department of Agriculture. This will require additional space, equipment and personnel, on the part of the Federal Government. Certainly in those areas where the public health service laboratories will continue to provide serotyping, this cooperation should continue. However, the Agricultural phase of disease control must provide additional service and information such as serotyping.

Phage typing, particularly for use in cattle infections, will be needed in the near future. A centrally located laboratory that could service all States would appear to be the logical place to develop this capability.

The Workshop also recommends that the epidemiology of the Salmonella and Arizona species be thoroughly surveyed and investigated. This can best be done by the official State regulatory personnel in cooperation with the U.S. Department of Agriculture official regularly assigned to that area. Certainly, an epidemiologist would contribute very valuable information to the knowledge and understanding of the disease producing ability of these two species. It seems obvious that clean feed (free of Salmonella) is one of the most important factors in our present and any future plans for control and eradication.

Each official State laboratory should survey the area facilities for confirming diagnoses and providing instruction and consultation for those laboratories wishing to improve their diagnostic technique. The Workshop endorses the laboratory procedures recommended by the ARS publication No. ARS 91-36 of October 1962, for isolating Salmonella organisms from animal feeds and meat byproducts, and the procedures for the isolation of Salmonella from animals as indicated in Miscellaneous Publication 739 of the National Poultry Improvement Plan and the National Turkey Improvement Plan.

It is further recommended that each State declare that infections with Salmonella or Arizona species be declared reportable diseases and that the identification of these species in feed, feed ingredients, feral birds, or

any animal be reportable. The reporting should be done immediately (48 hours) and not be withheld until all of the laboratory data have been filed.

Each State should provide its constituents with a suitable reporting form which should encompass the necessary statistical epidemiology of the particular case in question.

The U.S. Department of Agriculture should be charged with the responsibility of regularly compiling these statistics in order to inform the various States of the epidemiology of these two infections. The details regarding the individual cases can best be gathered after the laboratory records are complete. This will be done at the State level in cooperation with the Federal personnel assigned to that area.

The Workshop presents the above recommendations realizing that they are only a preamble to a future fully formulated eradication program.

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Dr. E. M. Himes
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Mr. Sam Moore

May 5, 1964

RECOMMENDATIONS OF THE WORKSHOP
ON
ANIMAL BYPRODUCTS AND FEEDS AND FISH MEAL

Since animal byproducts and feeds and fish meal are vehicles for transportation of Salmonella to livestock and poultry, the Workshop recognizes and/or recommends the following:

1. All Salmonella are pathogens and must be eliminated from animal feeds.

2. The production of Salmonella-free animal feeds is a desirable and attainable goal that should be achieved without undue delay.

3. The Workshop endorses the "Preliminary Recommended Sanitation Guidelines" for processors of industrial fishery products and for the processors of poultry and animal byproducts (recommended by the Agricultural Research Service, U.S. Department of Agriculture), and recommends that these guidelines be broadly distributed to these industries.

4. The development and adoption of similar guidelines (as in point 3) for the vegetable byproducts feed source industry and manufacturers of finished feeds.

5. The cooperative extension service publicize the proper handling of animal feeds on the farm to prevent contamination of feeds free of Salmonella.

6. Work is needed to evaluate soundness of guidelines regarding sampling techniques for detection of Salmonella in feed ingredients.

7. The development of specific procedures for cleaning and disinfecting preparation areas, storage areas, and transportation facilities handling animal feeds and feed ingredients.

8. Well-designed studies be made of "pasteurization" methods that will assure Salmonella-free finished animal feeds.

9. The development of specific procedures for rodent, bird, and insect control. Also, the use of air screens for fly control should be included as recommended procedure. Only products approved for use by the Pesticides Regulation Division, Agricultural Research Service, U.S. Department of Agriculture, should be applied.

10. The ultimate development of a regulation suitable for State and Federal enforcement to assure Salmonella-free animal feeds. This will also be applied to imported animal feed ingredients.

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May 5, 1964

# RECOMMENDATIONS OF THE WORKSHOP ON EPIDEMIOLOGY AND RESEARCH

The following recommendations apply to all domestic species, including poultry:

1. Reservoir hosts.

   A. Epidemiology: Make a complete investigation of outbreaks for potential reservoir, including man, feed, domestic animals and wild animals (vertebrate and invertebrate) with a definite established investigative procedure for the laboratory work with respect to both serology and culture. An identical epidemiological investigation should be conducted following a preliminary report of an outbreak, regardless of domestic animal involved.

   B. Research: A research part of this would be to make a similar survey on similar premises in the same general vicinity where there is no trouble with the disease as control premises.

2. Environment and transmission.

   A. Epidemiology: The epidemiological investigator should describe the environment prevailing prior to and at the time of discovery of the outbreak and attempt to assess its effect on the transmission.

   B. Research: It is recommended that research projects be established concerning the effect of environment on transmission, such as effects of humidity, crowding, chilling, shipping, etc.

3. Interspecies transmission.

   A. Epidemiology: The investigator would have to consider all the environment, etc., as mentioned above. Special attention should be paid to making a study of reservoir hosts in regard to the possibility that interspecies transmission has occurred. Cooperation of Public Health officials should be obtained in determining the inter-relation as far as humans are concerned.

   B. Research: Controlled research experiments are recommended to determine the effect of infection in one species upon another when they are put in close proximity. This would involve the introduction of an infected species among uninfected animals of a different species under known environmental conditions.

4. Interrupting the cycle of transmission.

   A. Epidemiology: The epidemiologist should be aware in his investigation of the necessity of finding a means of interrupting the cycle and to aim his investigation toward this end.

B. Research: Research should be directed toward determining the number of organisms necessary to establish infection, serological titer and carrier state. This research should be restricted to the major pathogens. Some research should be aimed at interrupting the cycle of transmission as may be possible through the fumigation of eggs.

Research is recommended to determine the effect of stress factors (natural or manmade) on transmission of the disease in livestock and these stress factors corrected as a means of breaking the cycle. These can be natural stress factors such as pregnancy, surgery, and shipment. Research into the role of the transporting vehicle in the spread of the disease is also recommended.

5. Relative host specificity.

A. Epidemiology: It is essentially the same as previous points.

B. Research: In respect to the subject matter of relative host specificity, additional research is needed to determine the susceptibility of the various hosts that might be encountered on farms where epidemics are occurring and to support work of an epidemiological nature.

6. Diagnostic agents.

A. Epidemiology: The epidemiologist should avail himself of the presently available diagnostic procedures, including serological and cultural techniques. This Workshop recommends that the U.S. Department of Agriculture, in cooperation with the various States, select a committee to review the entire area of procedures for serological and cultural techniques for the detection of Salmonella organisms in all species of domestic animals and make recommendations to the various States for the adoption of these procedures. The procedures to be recommended should apply to all classes of livestock and poultry, and their byproducts.

B. Research: It is recommended that research be expanded to include all diagnostic agents and techniques.

7. Immunization.

A. Epidemiology: Not applicable.

B. Research: It is not recommended that research be undertaken in regard to immunization for these different Salmonella, keeping in mind that this immunization will not interfere with eradication programs that are already in progress. It should not interfere with serological diagnosis of other infections, such as S. pullorum, S. typhimurium, S. gallinarum, S. choleraesuis, and S. dublin.

8. Application of phage to Salmonella control.

A. Epidemiology: Conduct field trials to prove the reliability and feasibility of phage typing in epidemiological investigations.

B. Research: It is recommended that research in phage typing should be undertaken to support any work of an epidemiological nature and that the Federal Government develop laboratory capability in the field of phage typing of Salmonella.

9. Disease eradication.

A. Epidemiology: It is recommended that pilot projects be undertaken to determine the feasibility of Salmonella eradication utilizing all of the epidemiological and research knowledge available from all sources.

GENERAL RECOMMENDATIONS:

1. We recommend that the Federal Government arrange to train State and Federal employees in epidemiological and diagnostic work (phage typing), that will further the control and eradication of Salmonella infections and call upon the various States that are willing to cooperate and are in a position to offer the necessary facilities, manpower, and funds to carry on such a program.

2. It is recommended that more effective liaison be established between epidemiological groups in the U.S. Public Health Service and the U.S. Department of Agriculture with special reference to Salmonella control.

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May 5, 1964

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